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Research Article

EXPERIMENTAL EVALUATION OF ANTIEPILEPTIC ACTIVITY OF METHANOLIC ROOT EXTRACT OF *GMELINA ARBOREA* LINN IN MICE

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ABSTRACT

The objective of the study was undertaken to investigate the antiepileptic activity of methanolic extract of *Gmelina arborea* linn Roots on Maximal electroshock induced seizures (MES), Pentylene tetrazole (PTZ), Picrotoxin (PIC) induced seizures , Biccuculine induced seizures in mice. It was found that the extract (200 & 400 mg/kg, p.o), significantly prolonged the onset of tonic seizures and reduced the duration of incidence of seizures in PTZ, PIC and Biccuculine induced seizure models where as in MES model, the extract showed significant effect in abolishing tonic hind limb extensions by inhibiting voltage dependant Na+ channels or by blocking glutaminergic excitation mediated by the N-methyl- D-aspartate (NMDA) receptor.

KEY WORDS: Antiepileptic activity, *Gmelina arborea*, Pentylene tetrazole, Picrotoxin, Bicuculine

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INTRODUCTION

Epilepsy is one of the most common of the chronic neurological disorder characterized by recurrent seizures of cerebral origin, presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness. The prevalence of epilepsy approximately 1% of the general population. The number of epilepsy patients in the whole of India about 10 million (in an estimated population of 1.0 billion).

The causes of epilepsy include chemical imbalance such as low blood sugar or sodium, head injuries, drug abuse or with-drawl, alcohol withdrawal, stroke or conditions that affect the blood vessels (vascular system) in the brain, hardening of the arteries (atherosclerosis) in the brain, brain tumour, brain infection, such as meningitis or encephalitis and Alzheimer's disease.[1]

Symptoms of epilepsy include aura,brief blackout followed by period of confusion, drooling or frothing at the mouth, eye movements, grunting and snorting, loss of bladder or bowel control, sudden falling, teeth clenching, temporary halt in breathing, uncontrollable muscle spasms with twitching and jerking limbs.[2]

India is regarded as the treasure trove of herbs in the world. Herbs demonstrate great versatility for the treatment of a broad variety of health needs. Medicinal plants are of great value in the field of treatment and cure of diseases. It has now been universally accepted that the herbal

medicines are far safer than that of synthetic medicines for curing of many of complex diseases.[3,4]

Gmelina arborea commonly known as white teak belongs to the family lamiaceae. It consists of Alakaloids,Flavonoids,Saponins,Steroids,Glycosides,Tannins, Benzoic acid etc. it is used as stomachic, galactagogue laxative and anthelmintic improve appetite, and useful in hallucination, piles, abdominal pains, burning sensations, fevers [4].

MATERIALS AND METHODS

Materials

Table 1: Materials used for the study

S.NO	NAME OF THE MATERIAL	NAME OF THE COMPANY
1	Diazepam	Calmpose, Ranbaxy Laboratories
2	Phenytoin	Sigma life sciences,Bangalore
3	Pentylenetetrazole	Sigma life sciences,Bangalore
4	Picrotoxin	Sigma life sciences,Bangalore
5	Bicuculline	Sigma life sciences,Bangalore
6	Poly ethylene glycol 400	Fludac, Cadila
7	Tween 80	
8	Nacl	
9	Electro Convulsometer	UGO BASILE

Animals

Albino mice of either sex weighing between 25-30 g were used in this study. All the animals were acclimatized in the quarantine room at Bhaskara Institutte of Pharmacy Animal house (BIPB,Bobbili,Vizianagaram), for 7 days and housed in groups of six under standard husbandry conditions like room temperature 23 ± 2^0c, relative humidity 30-70% and light/ dark cycle of 12 hours.

All the animals were fed with synthetic standard diet (National Institute for Nutrition, Hubsiguda, and Hyderabad) and water will be supplied *ad libitum* under strict hygienic conditions. All the experimental protocols were approved by Institutional Animal Ethical Committee (IAEC) of Andhra University. All the animal studies were performed as per the rules and regulations in accordance to the guidelines of CPCSEA.

All the animals were fasted 3h prior to oral administration of vehicle/standard/test compounds during the experiment. All the experiments were carried out during the light period (9:00 to 17:30h) to avoid circadian rhythm.

Preparation of methanolic root extract of Gmelina arborea

Soxhlet extraction technique was used to prepare ME of roots of *G. arborea* (ME). One hundred gram of powdered roots was packed in soxhlet extractor and extracted with methanol. After complete extraction, the ME was filtered and concentrated under reduced pressure by using rotary vacuum evaporator. The ME was dried in vacuum dryer and stored at − 20°C until used. The yield of the ME was found to be 28% w/w with respect to powdered root. The extract was prepared freshly in double distilled water at the time of administration to experimental animals.

Preliminary Phytochemical Screening

The methanolic root extract of *Gmelina arborea* was subjected for phytochemical screening for qualitative identification of phytoconstituents

Table 2: Phytochemical tests for methanolic root extract of *Gmelina arborea*

S.NO	PHYTOCHEMICAL CONSTITUENTS	INFERENCE
1	**Test for Carbohydrates**	
	Molisch's test	-
	Fehling's test	-
	Barfoed's test	-
	Benedict's test	-
2	**Test for Alkaloids**	
	Dragendorff's test	+
	Wagner's test	+
	Mayer's test	+
	Hager's test	+
3	**Test for Anthraquinone glycosides**	-
4	**Test for steroids**	
	Salkowski test	+
	Libermann Burchard	+
5	**Test for Flavonoids**	
	Shinoda test	+
6	**Test for Saponins**	
	Foam test	+
7	**Test for tannins**	+
8	**Test for glycosides**	+
9	**Test for triterpinoids**	-
10	**Test for benzoic acid**	+

+ indicates presence; - indicates absence

Experimental design

Acute Oral Toxicity Study

The methanolic extract of *Gmelina arborea* roots was administered orally to different groups of mice at different dose levels and found to be safe even up to the dose level of 2000 mg/kg, did not produce any mortality or toxic symptoms. The survived animals were sacrificed.Hence, $1/5^{th}$ and $1/10^{th}$ of Maximum Tolerance Dose (2000 mg/kg; p.o) were selected for the present study.[5]

METHODS

Maximum Electric Shock Induced Seizures Model (MES)[6]

In this model, Animals were divided into 4 groups of 5 animals each weighing between 25-30gms.

Group I – Control (Distilled water 10 ml/kg, p.o)

Group II – Standard (Phenytoin 25 mg/kg, p.o)
Group III-Low dose of MEGAR (200 mg/kg, p.o)
Group IV-high dose of MEGAR (400 mg/kg, p.o)

The low and high doses of extract were administered to groups of mice orally, 60mins before application of electric shock (30mA/56 Hz for 10 sec) using corneal electrodes. The duration of hind limb tonic extension was noted.[7]

Pentylene Tetrazole Induced Seizure Model (PTZ)

In this model, Albino mice of either sex weighing between 25-30g were divided into four groups of five mice in each, were fasted for 3h prior to the test but water will be supplied *ad libitum*.

Group I - Control (Distilled water 10 ml/kg, p.o)
Group II - Standard (Diazepam, 10 mg/kg, p.o)
Group III - Low dose of MEGAR (200 mg/kg, p.o)
Group IV - high dose of MEGAR (400 mg/kg, p.o)

The control, standard and test groups were pretreated with distilled water (10 ml/kg, p.o), diazepam (10 mg/kg, i.p) and plant extract (low & high doses) respectively. PTZ (80mg/kg, i.p) was injected to the control and extract treated groups after one hour and diazepam treated group after 30mins. The animals were placed in observational cages; the latency and duration of myoclonic jerks as well as incidence of seizures were recorded for 30mins after PTZ treatment. Time taken for death/recovery was noted.[8]

Picrotoxin Induced Seizures Model

In this model, Animals were divided into 4 groups of 5 animals each weighing between 25-30g.

Group I - Control (Distilled water 10 ml/kg, p.o)
Group II - Standard (Diazepam, 10 mg/kg, p.o)
Group III - Low dose of MEGAR (200 mg/kg, p.o)
Group IV - high dose of MEGAR (400 mg/kg, p.o)

In this test, all the animals were fasted for 3h prior to the experiment and were treated as above. Picrotoxin (6 mg/kg, i.p.) was used to induce seizure. Animals were treated with dose of 2 low and high doses p.o respectively, 30mins before picrotoxin administration. Diazepam (10 mg/kg, p.o) was used as a standard drug. Seizure stage and seizure latency were the two parameters used to evaluate antiepileptic activity of the drug.[9]

Bicuculline Induced Seizures

In this model, Animals were divided into 4 groups of 5 animals each weighing between 25-30g.

Group I - Control (Distilled water 10 ml/kg, p.o)
Group II - Standard (Diazepam, 10 mg/kg, p.o)
Group III - Low dose of MEGAR (200 mg/kg, p.o)
Group IV - high dose of MEGAR (400 mg/kg, p.o)

In this test, all the animals were fasted for 3h prior to the experiment and were treated as above. Bicuculline (18 mg/kg, i.p.) suspended in 0.5ml of Tween 80 and adjusted to an appropriate volume with physiological saline, was used to induce seizure. Animals were

treated with dose of 2 low and high doses p.o respectively, 30mins before Bicuculline administration. Diazepam (10 mg/kg, p.o) suspended in minimum amount of Polyethylene glycol was used as a standard drug. Duration of seizures and seizure latency were recorded to evaluate antiepileptic activity of the extract.[10]

Statistical Analysis

Results were presented as Mean ± SEM. The data was subjected for statistical analysis by One way analysis of variance (ANOVA) followed by Dunnett's t test and $P<0.05$*, 0.01** and 0.001*** were considered as significant, $P>0.05$ was considered as non-significant (ns) Vs Control group.

Table 3: Effect of *Gmelina arborea* on duration of hind limb extension in Maximum Electric Shock induced model

S.No.	Treatment groups	Duration of Hind limb Extension (sec)	No. of animals Extensed/No. of animals used
1	Control	11.6 ± 0.50	5/5
2	Phenytoin (25mg/kg)	0 ± 0**	0/5
3	MEGAR (200 mg/kg)	5.8 ± 0.86**	2/5
4	MEGAR(400 mg/kg)	0 ± 0**	0/5

n=5 in each group. Significance at $P<0.05$*,$P<0.01$**.

Fig 1: Effect of *Gmelina arborea on* duration of hind limb extension in Maximum Electric Shock induced model

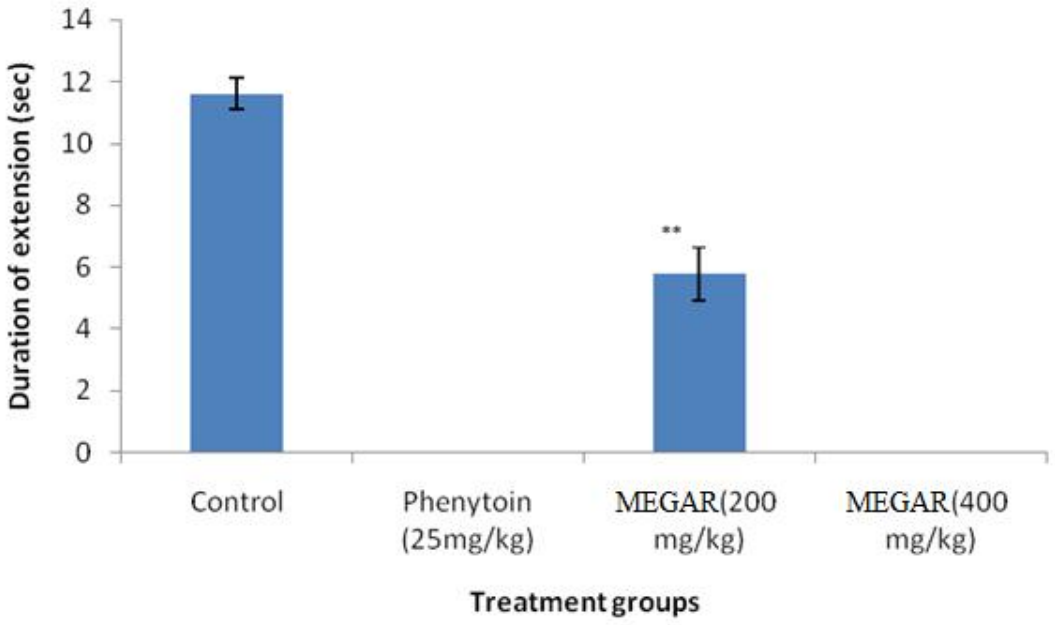
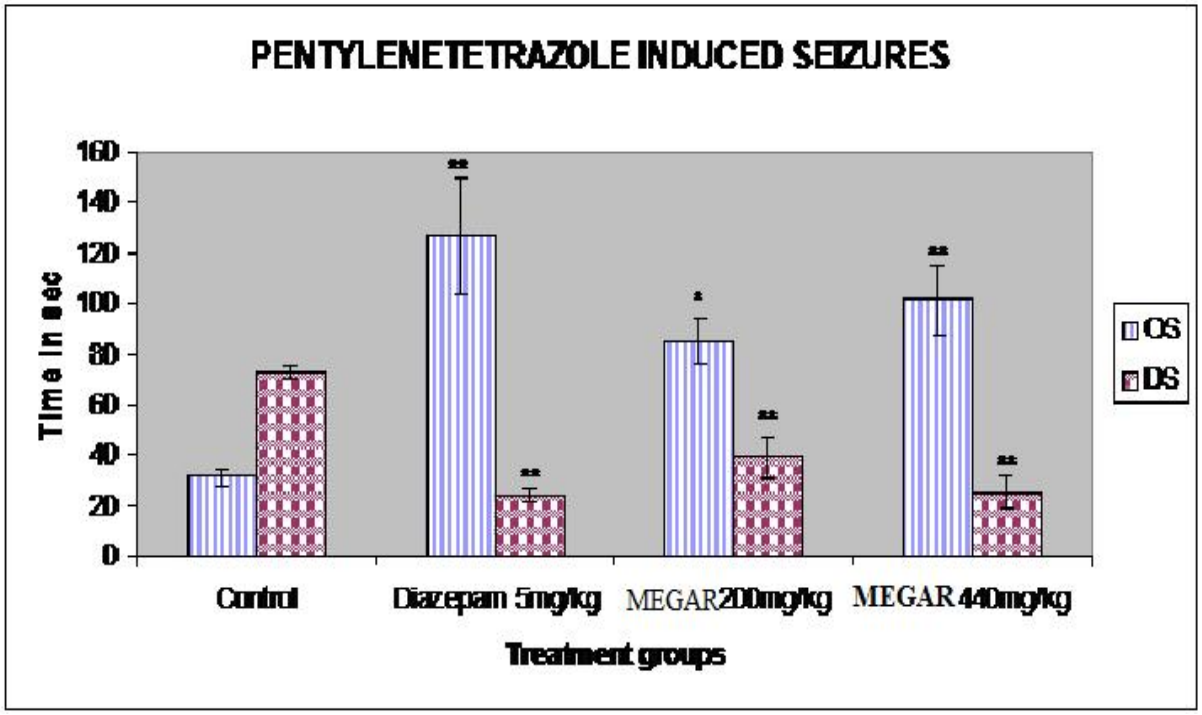


Table 4: Effect of *Gmelina arborea* on duration and onset of seizures in Pentylenetetrazole induced seizures in mice

S.NO	TREATMENT	ONSET OF SEIZURES (Sec)	DURATION OF TONIC CLONIC CONVULSIONS (Sec)	% MORTALITY D/N	% PROTECTION
1	Control	31.6 ±3.31	72.6±2.75	5/5	0 %
2	Diazepam(5 mg/kg,P.O)	126.8±23.27**	24.4 ±2.52**	0/5	100 %
3	MEGAR (200mg/kg , P.O)	85.6 ± 9.27*	39.4± 8.68**	1/5	80 %
4	MEGAR (400mg/kg,P.O)	101.8±13.45**	25.2 ± 6.78**	0/5	100 %

n=5 in each group. Significance at $P<0.05$*, $P<0.01$**, $P<0.001$** *

Fig.No.2: Effect of Gmelina arborea on duration and onset of seizures in Pentylenetetrazole induced seizures in mice



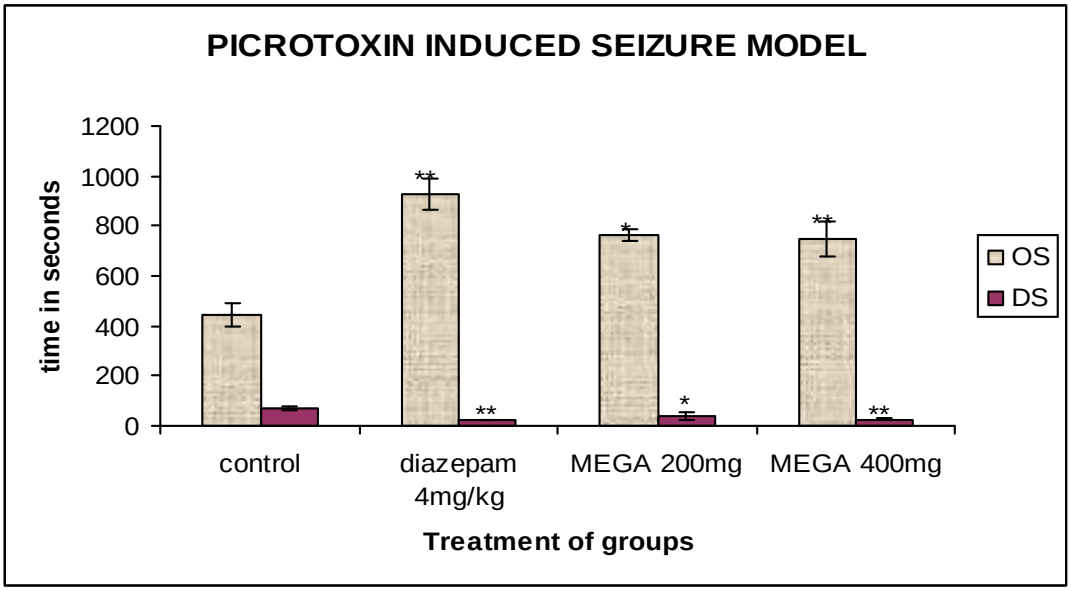
MEGAR =Methanolic extract of *Gmelina arborea* .L roots, OS= Onset of seizures, DS = Duration of seizures, Significance at *$P<0.05$, **$P < 0.01$ using Statistical analysis by one-way ANOVA followed by dunnet's *t*-test

Table 5: Effect of *Gmelina arborea* on onset and duration of seizures in Picrotoxin induced seizures in mice

S.NO	TREATMENT	ONSET OF SEIZURES (Sec)	DURATION OF TONIC CLONIC CONVULSIONS (Sec)	% PROTECTION
1	Control	443.4±47.328	326.6±63.48	0 %
2	Diazepam (10 mg/kg,I.P)	928.2±47.328**	12.4±0.67**	100 %
3	MEGA (200mg/kg , P.O)	673.2±37.49 *	53.27±12.78**	60 %
4	MEGA (400mg/kg,P.O)	749.8±69.04**	13.6±1.69**	100 %

n=5 in each group.Significance at *P*<0.05*,*P* <0.01**, *P* <0.001*** .

Fig. No. 3: Effect of Gmelina arborea on onset and duration of seizures in Picrotoxin induced seizures in mice



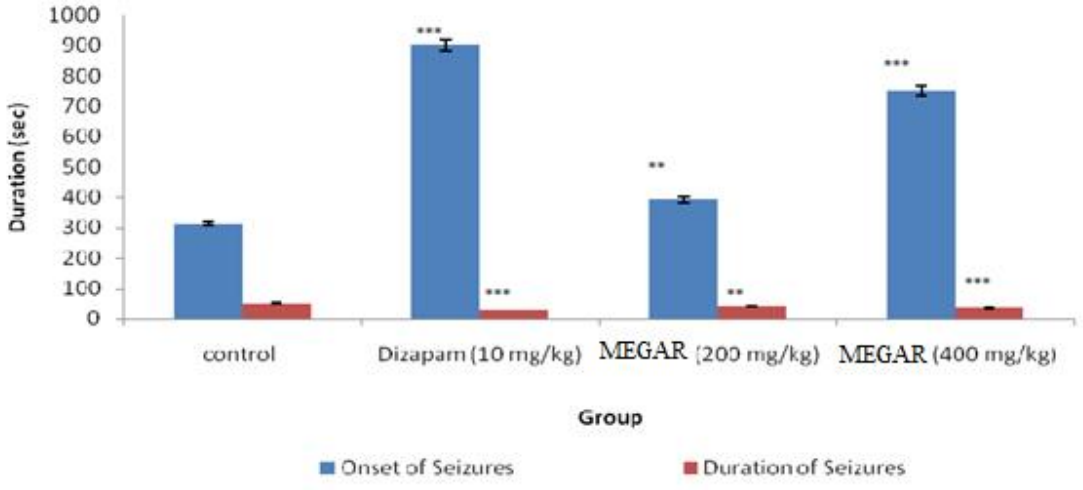
MEGAR = Methanolic extract of *Gmelina arborea* .L roots , OS= Onset of seizures , DTCC = Duration of tonic clonic convulsions, Significance at **P*<0.05, ***P* < 0.01 using Statistical analysis by one-way ANOVA followed by dunnet's *t*-test

Table 6: Effect of *Gmelina arborea* on duration of seizures in Biccuculine induced seizures in mice

S.No.	Treatment groups	Onset of Seizures (sec)	Duration of Seizures (sec)	% Protection
1	Control	312.4 ± 4.697	50.6 ± 1.72	0%
2	Diazepam (10 mg/kg)	901.2 ± 16.945***	28.6 ± 1.030***	100%
3	MEGAR (200 mg/kg)	393.6 ± 10.856**	40 ± 1.517**	20%
4	MEGAR (400 mg/kg)	750.8 ± 17.273***	34.6 ± 1.965***	80%

n=5 in each group. Significance at $P<0.05$*,$P<0.01$**, $P<0.001$***

Fig. No .4: Effect of *Gmelina arborea* on duration of seizures in Biccuculine induced seizures in mice



RESULTS

Effect of MEGAR on MES-induced convulsions in mice

The data has been shown in Table 3. In this MES model, MEGAR at (200 mg/kg, p.o) were showed reduced duration of tonic hind limb extension and the standard drug Phenytoin (25 mg/kg, p.o) and MEGAR (400 mg/kg, p.o) treated animals exhibited abolished hind limb extension.

Effect of MEGAR on PTZ-induced convulsions in mice

The data has been shown in Table 4. In this test, MEGAR at both doses (200 mg/kg & 400 mg/kg) exhibited significant increase in onset time and decrease in duration of tonic-clonic seizures. The two doses offered a protective effect of 80% and 100%. Standard drug

Diazepam (5 mg/kg) has exhibited a significant anticonvulsive activity and offered 100% protection when compared to control.

Effect of MEGAR on picrotoxin induced convulsions in mice

The data has been shown in Table 5. In this test, MEGAR at both doses (200 mg/kg & 400 mg/kg) exhibited significant increase in onset time and decrease in duration of tonic-clonic seizures. The two doses offered a protective effect of 80% and 100%. Standard drug Diazepam (10 mg/kg) has exhibited a significant anticonvulsive activity and offered 100% protection when compared to control.

Effect of MEGAR on Bicuculline induced convulsions in mice

The data has been shown in Table 6. In this model, MEGAR at dose (200 mg/kg,p.o & 400 mg/kg,p.o) exhibited significant increase in onset time and decrease in duration of hind limb extension. The Standard Diazepam (10 mg/kg, p.o) profoundly abolished the onset and duration of Biccuculine induced seizures.

DISCUSSION

There are a number of synthetic antiepileptic drugs currently available for use in the management, control and treatment of individuals with epilepsy. However, most of the synthetic drugs are not only inaccessible and unaffordable, but also possess many toxic adverse effects. There is, therefore, a dire need for the development of cheap, effective and safe antiepileptic agents from plants and other sources.[11]

In ayurvedic literature, there are reports regarding the use of *Gmelina arborea* in treating epilepsy. However it remains far from establishing its antiepileptic activity by pharmacological methods. Hence Gmelina arborea was selected to evaluate its antiepileptic activity in animal models.[12]

MES is also one of the commonly used models for preliminary testing of anticonvulsant drugs that produces generalized tonic-clonic seizures. i.e. hind limb tonic extensor (HLTE), tonic flexion and clonic convulsions. In untreated animals a single MES produced an immediate tonic hind limb extension for 5-10 sec duration followed by clonic seizures by inhibiting voltage dependant Na+ channels (Phenytoin, Valproate) or by drugs that block glutaminergic excitation mediated by the N-methyl- D-aspartate (NMDA) receptor.[13,14]

Prevention of PTZ induced seizures in laboratory animals is the most commonly used preliminary screening test for characterizing potential anti-convulsant drugs. The test is assumed to identify anticonvulsant drugs effective against generalized clonic seizures, as PTZ produces clonic and tonic convulsions.. The mechanism by which PTZ exert its convulsant action is by acting as an antagonist at the $GABA_A$ receptor complex. Drugs offer protections against tonic–clonic seizures induced by PTZ are considered to be useful to control myoclonic and absence seizures in humans. Various factors like age, sex, species, diet, water, day/light cycle, temperature, preparation dose and route of administration are known to affect the response of the animal to PTZ induced seizures. Diazepam acts through the activation of $GABA_A$ receptors and facilitate the GABA mediated opening of chloride channels.[15,16]

Picrotoxin a GABA-receptor antagonist produces seizures by blocking the chloride ion channel linked to $GABA_A$-receptors, thus preventing the entry of chloride ions in to the brain. This process will in turn inhibit GABA neurotransmission and activity in the brain.

Phenobarbitone and diazepam are believed to enhance GABAergic neurotransmission by increasing chloride ion flux through chloride ion channel at GABA-receptor sites.[17]

Biccuculine is competitive antagonist of ionotropic GABA-$_A$ receptors. The action of bicuculline is primarily on the ionotropic $GABA_A$ receptors, which are ligand-gated ion channels concerned chiefly with the passing of chloride ions across the cell membrane, thus promoting an inhibitory influence on the target neuron.[18,19]

CONCLUSION

MEGAR at doses of 200mg/kg, p.o and 400mg/kg, p.o showed significant delay in onset of tonic convulsions and decreased the duration of seizures in PTZ, PIC and Biccuculine induced seizures. The present investigation suggests that the methanolic extract of Gmelina arborea may possess antiepileptic activity against PTZ, PIC, Biccuculine induced seizures by enhancing GABA inhibitory neurotransmission and MES, it blocks seizures spread and tonic extension either by inhibit voltage dependant Na+ channels or by blocking glutaminergic excitation mediated by the N-methyl- D-aspartate (NMDA) receptor.[20,21]

The confirmation of Phytochemical screening gave positive results for alkaloids and flavonoids which may be the active constituent responsible for the antiepileptic activity of Gmelina arborea.

Further investigation should be carried out to isolate and identify the chemical constituent which is responsible for its antiepileptic activity.

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